PATENT COOPERATION TREA . /

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 December 1999 (14.12.99)	
International application No. PCT/IB99/00924	**Applicant's or agent's file reference** CP/FP966
International filing date (day/month/year) 21 May 1999 (21.05.99)	**Priority date** (day/month/year) 23 May 1998 (23.05.98)
Applicant MCLAREN, James	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

24 November 1999 (24.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Juan Cruz

Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TREAT

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
ARMENGAUD, Alain
Cabinet ARMENGAUD AINE
3, avenue Bugeaud
F-75116 Paris
FRANCE

Date of mailing *(day/month/year)* 20/08/1999	
Applicant's or agent's file reference CP/FP966	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/IB 99/00924	International filing date *(day/month/year)* 21/05/1999

Applicant

DANSTAR FERMENT A.G. et al.

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Heike Zoglauer

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

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REC'D 12 JUL 2000

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CP/FP/VB59754-966		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB99/00924	21/05/1999	23/05/1998
International Patent Classification (IPC) or national classification and IPC C12P7/06		
Applicant DANSTAR FERMENT A.G. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Auth rity (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 15 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI	☒	Certain documents cited
VII	☒	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/11/1999	11.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Douschan, K Telephone No. +49 89 2399 8702

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB99/00924

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1,3,4,6,8,9, 18-24	as originally filed	
2,5,7,10-17,25	with telefax of	31/05/2000

Claims, No.:

1-19	with telefax of	31/05/2000

Drawings, sheets:

1/2,2/2	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. R asoned statement und r Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1 - 19
	No:	Claims
Inventive step (IS)	Yes:	Claims 1 - 19
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1 - 19
	No:	Claims

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) **Novelty - Art. 33 (1) and (2) PCT** :

Claims 1 to 19 meet the requirements for novelty since the prior art does not disclose a process for alcoholic fermentation using two microorganisms, one fermentation organism and a mineral-rich or mineral-enriched yeast as a nutrition source for the fermentation organism.

2) **Inventive Step- Art. 33 (1) and (3) PCT** :

Similarly, it is considered that claims 1 to 19 meet the requirements for inventive step, since the prior art does not disclose or suggest a process for alcoholic fermentation using two microorganisms as detailed above. The comparative test data supplied in the description reveal that supplying the fermentation microorganism with minerals through a second mineral-rich or mineral-enriched microorganism (yeast) gives rise to a surprising increase in the fermentation activity when compared to supplying the wort with minerals and microorganisms (yeast) either separately or together.

Re Item VI

Certain documents cited

The two patent references have been inserted as prior art references into the description, the textbook excerpt has been introduced by the applicant.

US 4840802 A

JP 63287474 A

KUNZE: 'Technology, Malting and Brewing', , VERSUCHS-UND-LEHRANSTALT FüR BRAUEREI, BERLIN

Re Item VII

Certain defects in the international application

(a) On page 14, line 10 the formulation: "per litre" is not correct in connection with "ppm".

(b) In Claim 15 the term: "fermentation microorganism holding vessel" appears twice. It seems that the term "propagating vessel" was intended.

(c) The term: "obtainable" in Claim 3 does not characterise the subject-matter properly. The term "obtained" appears to be more appropriate.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12P 7/06, C12C 11/00, C12G 1/00, 3/00, 3/02, C12N 1/16, 1/18, C12C 11/02

A1

(11) International Publication Number: WO 99/61646

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(30) Priority Data:
9811103.2 23 May 1998 (23.05.98) GB

(71) Applicant *(for all designated States except US)*: DANSTAR FERMENT AG [CH/CH]; 12, Alpenstrasse, CH–6301 Zug (CH).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: MCLAREN, James [GB/FR]; La Bourdeneuve de Lavat, F–32700 Lectoure (FR).

(74) Agents: ARMENGAUD, Alain et al.; Cabinet Armengaud Ainé, 3, avenue Bugeaud, F–75116 Paris (FR).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: IMPROVED PROCESS FOR ALCOHOLIC FERMENTATION

(57) Abstract

The invention relates to a process for alcoholic fermentation, comprising the use of at least one mineral–rich or mineral–enriched yeast as a nutrient source for said fermentation.

FOR THE PURPOSES OF INFORMATION ONLY

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IMPROVED PROCESS FOR ALCOHOLIC FERMENTATION

The present invention generally relates to an improved process for alcoholic fermentation which comprises the use of a mineral-rich or mineral-enriched yeast as a nutrient in said fermentation process, and to the use of such a yeast as a nutrient in an alcoholic fermentation process.

There are a number of minerals that are required in trace amounts for efficient alcoholic fermentation. These in particularly include metals capable of alterating the fermentation metabolism, such as divalent metals *e.g.* manganese, magnesium and zinc. There has been an increasing awareness of the importance of such trace minerals in alcoholic fermentations, particularly with respect to beer.

The zinc concentration of a wort is of particular importance from two perspectives. Firstly, if limiting, it can lead to sub-optimal, even incomplete fermentations, problems with head retention and yeast flocculance. Secondly, adequate levels of zinc can aid in the optimisation of alcoholic fermentations, vis a vis ethanol production and fermentable sugars uptake. This second perspective has a greater importance during fermentations when the yeast is subject to greater stresses. Moreover, traditionally, breweries recycle their yeast from one fermentation to another. Repitching yeast from one fermentation where the zinc is limiting

into another beer wort, which is also deficient, would exacerbate fermentation problems.

Other minerals have been shown to be of importance in the course of an alcoholic fermentation. Manganese is thus known to be implicated as a substitute zinc metabolism, and could possibly mitigate some toxic effects associated with high concentrations of zinc. Another example is magnesium which is reported to be important for alcohol efficiency in fermentations. This is particularly a problem for the fermentation of certain substrates where there is an excess of calcium ions present. Calcium is indeed known to be antagonistic to magnesium metabolism and, for example, in beer, calcium is deliberately added in order to control the pH (acidity) and activate some of the enzymes of the malted barley.

For most alcoholic fermentations, there is thus a perceived natural mineral deficit in the substrate, and minerals, in the form of mineral salts such as zinc/manganese/magnesium chloride or sulphate, are generally added directly into the substrate, *e.g.* into the wort at the boiling stage for beer production. The use of such mineral salts, whilst relatively effective, conflicts with the desire by some industrialists to produce additive free alcohols.

Alternatives to the addition of mineral salts have thus been proposed in the past decades. These include pre-loading the fermentation yeast with a metal in such a way that the metal is hardly released from the cell body of the fermentation yeast during the fermentation process, or using ash trub or acid extracts of spent grains or hop trub so as to make use of the trace elements they contain. But all these alternatives are, in terms of quantity and quality of alcoholic fermentation production, at the best only substantially equivalent to the initial solution of directly adding mineral

salts. Their industrial application is thus quite restricted, and some of them even show problems of off-odours associated with the process (*e.g.* acid extract use). None of the prior art techniques thus provides with fully satisfactory results.

It is an object of the present invention to provide with an improved process for alcoholic fermentation which, further to not showing the drawbacks of prior art techniques, is quantitatively, and also qualitatively more efficient than the solution of directly adding mineral salts or any other alternative solution. The process and use according to the invention allow an enhanced fermentation yeast growth, and accelerated fermentation. It also shows many advantages : it is very easy to handle, it applies to any alcoholic fermentation process, and is economically very beneficial. The process of the invention comprises the use of at least one mineral-rich or mineral-enriched yeast not as fermentation micro-organism, but as a nutrient source : the present invention indeed shows that, contrary to the received wisdom in the brewing industry, another micro-organism can be efficiently added to a fermentation process without leading to microbial instability, and that it is moreover able to provide the fermentation micro-organism with nutrients, and particularly with minerals such as zinc, magnesium, manganese in a very efficient way. As will be further described and illustrated below, this efficiency as a mineral source not only lies into an efficient mineral flux from the yeast(s) used as a nutrient source towards the fermentation micro-organism : the process and use according to the invention are indeed more efficient than the direct addition into the substrate of an equivalent quantity of mineral salt, and is

even more efficient than the separate addition of both mineral salt on one hand, and a dead yeast on the other hand (see examples). That is to say, the process according to the invention shows synergetic effects in terms of mineral nutrition. These synergetic effects may at least partially lie in an increased mineral bioavailability favorable to the fermentation micro-organism.

The term "fermentation process" is herein meant as including the whole production process, and is no way limited to the precise biological step of fermentation. It *e.g.* also includes the fermentation yeast propagation step and the process of production of the substrate. The term "nutrient" herein comprises any element which can be considered of nutritive value to the fermentation micro-organism, and thus also comprises micro- or trace nutrients. It has to be also pointed out that the word "yeast" is herein meant as a yeast cell which can be living or dead, and which still comprises at least one structure corresponding to an insoluble cell structure.

A preferred yeast for use according to the invention is a mineral-enriched yeast. Said at least one mineral-rich or mineral-enriched yeast is advantageously chosen among the food grade yeast genera. Examples of appropriate yeasts include the *Saccharomyces* genera (*e.g. Saccharomyces cerevisiae*) and the *Kluyveromyces* genera.

In an embodiment of the present invention, said at least one mineral-rich or mineral-enriched yeast is, before use, such as obtained by adding about 1,000 to about 200,000 ppm (relative to the weight of the yeast, as measured on a dry weight basis) of a salt of said mineral to a live culture of yeast at a temperature of about 4-40°C, preferably about 25-32°C, at a pH of between about 3.5 to about 7.0, preferably about 4.6 to 6.6, for a

period of about 1-20 or 24 hours, preferably 2-16 hours so as to allow said yeast to incorporate, absorb and/or adsorb the mineral(s). Any salt, *e.g.* acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide, perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate or valerate is appropriate. Comparative assays can be performed by the person skilled in the art to determine the most efficient mineral source. Said mineral incorporation can correspond to an absorption and/or an adsorption. When incorporated, said mineral may remain as a mineral and/or be transformed into a salt and/or an organic form. It has to pointed out that the efficacy of the use according to the invention is not necessarily directly and solely dependent on the resultant mineral concentration of the substrate : bioavailability has also to be taken into account. In another embodiment, said at least one mineral-rich or enriched yeast is a commercially available product, *e.g.* a product from the Danstar Ferment A.G. Mineral Enriched Yeast range.

Said yeast is advantageously rich in, or enriched in at least one mineral which is capable of altering the metabolism of an alcoholic fermentation. A capacity of altering the metabolism of an alcoholic fermentation can be easily assessed by the person skilled in the art, *e.g.* by comparing the growth level of the fermentation micro-organism, and/or the rate of fermentation, and/or the secondary metabolites concentrations and/or the flavour profile, in the presence and in the absence of the mineral candidate under standard appropriate laboratory conditions. The word "mineral" herein also comprises oligoelements. Such a mineral is preferably a metal, and most preferably a divalent metal. It is advantageously chosen among the group consisting of zinc, magnesium,

manganese. A most preferred mineral is generally zinc. But when it deals with negating the repressive effect of the calcium and thereby increasing sugar/alcohol conversion, magnesium is then preferred. Said at least one mineral-rich or enriched yeast can carry more than one nutrient mineral at a time, *i.e.* it can be a combination or a permutation of *e.g.* magnesium and zinc.

The use of said at least one mineral-rich or enriched yeast according to the invention is such that the mineral(s) contained therein or thereon is(are) released to the benefit of the fermentative micro-organism culture. Preferably, said at least one mineral-rich or enriched yeast contains, before being used, a concentration ranging from about 1.000 to about 200.000 ppm for each mineral it carries.

One of the many advantages of the process and use according to the invention lies in the fact that said at least one mineral-rich or enriched yeast can be supplied in any form appropriate to the precise fermentation process wherein it has to be used. It can be supplied in a living form, or in a dead form. It may be cellularly intact, but, as it is used as a nutrient source, and not for a cell production, it also can be cellularly slightly ruptured.

Said at least one mineral-rich or enriched yeast can indeed be used under a variety of forms which include a dry form, a liquid form, a frozen form, a freeze-dried form, a paste, or a powder. It may have been sterilised or not. It may be used on its own or as part of a mixture of other products. The process and use according to the invention can thus be seen as the use of at least one sacrificial yeast as a nutrient source in alcoholic fermentations.

Another advantage lies in the fact that said use can be performed, as desired, at any step of the fermentation process. A simple direct addition of said at least one mineral-rich or enriched yeast at at least one step of the fermentation process is efficient. It may thus be added directly into the boiling vessel, and/or the fermenter, and/or any vessel between the two, and/or into the vector micro-organism holding or propagating vessels. For example, in beer production, the addition to the wort can be performed during alcohol production process or fermentation micro-organism propagation process, before or after boiling.

Said at least one mineral-rich or enriched yeast can thus be added directly to the wort so that it is killed during the wort boiling stage. It may be also added to the cooled wort prior to, during or after yeast pitching. Preferably said at least one mineral-rich or enriched yeast is added to the boiling wort. Advantageously, said use according to the invention is performed so that it leads to an increase of at least about 0.05 ppm of the mineral content of the substrate of said fermentation . The fermented substrate itself may be distilled or not.

The use according to the invention is particularly efficient in that it accelerates alcoholic fermentation velocity greater than when the mineral concentration is raised by the addition of the equivalent concentration of mineral when derived from a mineral salt. A synergetic effect can moreover be outlined when comparing to the addition of mineral salt on one hand and dead yeast on the other hand (see *e.g.* laboratory tests 2 and 3 of example 1 for zinc). The fermentation duration needed therefore decreases (see examples below). The limit to primary fermentation is achieved faster, significantly in comparison to when the equivalent

mineral concentration is derived from a mineral salt. The number of hours necessary to achieve the standard specific gravity (about 3.6°P or about 3.8°P for beer) is decreased: the time needed to achieve the attenuation degree of fermentation is decreased of several hours (about 20 hours in the below examples with zinc).

The use according to the invention also allows the fermentation to progress to absolute dryness, *i.e.* to an absence of residual fermentable sugars in the alcohol thus produced (see *e.g.* the below example 2). It not only allows a higher production of alcohol, but also a qualitatively better one (see *e.g.* the below example 1). And last, but not least, despite said fermentation acceleration, the alcohol produced according to the invention tastes equal to or better, in comparison to when the equivalent mineral concentration is derived from a mineral salt (see also the below example 3).

The process and use according to the present invention are of first interest for the beer industry, but it can also apply to any alcoholic fermentation beit cereal based, such as whisky or sake as well as fruit, sugar or honey based fermentations, such as wine, brandy, cider, fruit wines, mead, rhum, tequila, industrial alcohols, potable alcohols, vodka, gin, etc.

The present invention also relates to the use of at least one mineral-rich or mineral-enriched yeast as a nutrient source as herein described for the production of alcohol by a fermentation process.

Technical features and advantages of the present invention are herein further illustrated by several examples, which are given for illustration purposes and are in no way intented in restricting the scope of the invention. In these examples, reference is made to :

- figure 1 which represents the results of brewery trial 1 (Extracts [°Balling] as a function of the number of fermentation days), and to

- figure 2 which represents, in a similar way as for figure 1, the results of brewery trial 2.

In figures 1 and 2, the legend is the following :

lozenges : O-wort

squares : zinc yeast

horizontal line : primary attenuation limit

EXAMPLE 1: FERMENTATION SPEED

Three laboratory tests and two brewery trials were carried out to show the relative effectiveness of sacrificial zinc yeast and zinc chloride addition to worts containing different natural concentrations of zinc, and then fermented by yeasts containing different natural concentrations of zinc.

Materials and Methods

Materials

Yeast strains and provenance (laboratory tests)

In the results herein reported, the yeast sources were as follows :

The yeast strain used in all laboratory trials was a strain of S. cerevisiae (lager type) obtained from four different commercial breweries in Germany. The sample taken was from their stock designated to be used for their next fermentation. The strain is for tests one, two and three, and

in brewery trial 2 is deposited with the Technical University of Munich-Weihenstephan Hefebank; and designated as strain number W 34/70. The strain used in brewery trial 1 is deposited with the Technical University of Munich-Weihenstephan Hefebank, and designated as strain number W 120.

The yeast used for all laboratory trials was obtained, when needed, in the form of a cream from the appropriate brewery. The cream was centrifuged in a SORVAL RC5B centrifuge at 2700g for 10 minutes and the supernatant was discarded. The yeast paste was weighed and resuspended in cooled wort, aerated and then added directly to the fresh worts. The zinc content of the yeast was measured before pitching.

Media

Laboratory Test Fermentations

The wort used was obtained from three commercial breweries in Germany. In test number one the wort is used to make their "helle" type beer. In test number two the wort is used to make their "festbier". In test number three the wort is used to produce "pilsner" type beer. In all cases the wort was collected at the end of the boil and was therefore hopped to the normal level of that product for that particular beer. The worts had not been treated in the respective brewery in any way to alter the natural level of zinc. The worts were boiled for fifteen minutes before being cooled to the fermentation temperature, 10°C, and pitched with fermentation yeast.

Brewery Trials

Two brewery trials were carried out in two commercial breweries in Germany. The brewery in trial one was the Brauerei Kreiger, 944505 Landau, D. Isar, Bavaria, Germany. During a normal commercial production phase, two consecutive "helle" type worts, number 22 and 23, produced on 20th and 21st of April 1999 respectively, were designated for experimental observation. They were produced from the same recipe, one immediately after the other, from the same malt and hop stocks, and brewing water. 110 hectolitres of wort was collected from each brew.

The brewery in trial two was, the Privatbrauerei Kitzmann, Kitzmann Bräu KG, Südliche Stadmauerstrasse 25, 91954 Erlangen Bavaria, Germany. During a normal commercial production phase, one wort, designated brew number 120, produced on the 26th of April 1999, containing 286 litres of wort at 11.6° Balling, was separated into two fermenters each containing one hundred and forty-three hectolitres of "pilsner" wort. Both temperature profiles of the fermentations were as per normal for that brewery for that beer type.

Zinc Preparation

Laboratory tests

Mineral zinc, when used, was added in the form of the salt zinc chloride. This salt is used extensively by breweries throughout the World.

Zinc measurements on the wort and yeast samples were carried out by atomic absorption spectrometry as per the MEBAK standard brewery analytical procedures, see *e.g* Lutz,A.: Bestrmmung, Vorkommen und

Verhalten von Kontaminationen durch verschiede umweltrelevante Spurenelemente in Bereich der Brauerei, Dissertation TU München, (1996), S21 ff.

Preparation of Sacrificial zinc Yeast.

A sacrificial zinc yeast can be prepared by the person skilled in the art by any method convenient for producing a zinc-rich yeast. Standard methods use the incorporation, absorption and/or adsorption of zinc by the yeast. It should be noted that to implement the present invention, the yeast could be living or dead.

The preparations used in the trials herein reported were produced in some manner as per the following method.

Zinc, at a concentration of between 1,000 and 200,000 ppm (relative to the weight of the yeast or yeast fraction, as measured on a dry weight basis), in the form of zinc sulphate, chloride, acetate, phosphate, or some other appropriate zinc form is added to a live or dead culture of *S. cerevisae* at a temperature of about 4 to about 40° Celsius (preferably of about 25-32°C) at pH of between about 3.5 to about 7 (preferably about 4.6 to about 6.6), for a period of 1 to 20 or 24 hours so as to allow the culture to incorporate, absorb and/or adsorb the zinc.

Two base dry zinc yeast preparations were used in the trials;

Preparation one contained 10,500 ppm mineral zinc

Preparation two contained 70,000 ppm mineral zinc

Other zinc yeast preparations are also commercially available from Danstar Ferment AG, 20 Alpenstrasse, 6301 ZUG, Switzerland (MEY Zn 50).

All the zinc preparations were added to the boiling worts, at the start of boiling.

In summary, the state of zinc in the yeasts and worts in the three laboratory tests and two brewery trials is as follows in :

Table 1

	Test 1	Test 2	Test 3	Trial 1	Trial 2
Wort ppm	0.2	0.06	0.1	0.06	0.1
Fermentation Yeast mg/100g dry	5.9	1.3	4.1	1.3	5

Fermentation Yeast Preparation

A portion of the test yeast, 30 g of the paste, was re-suspended in 250 millilitre of boiled wort and aerated for approximately five minutes by way of a magnetic stirrer. The yeast preparation was then divided into seven equal aliquots and pitched into the appropriate test wort.

Brewery trial one and brewery trial two were conducted similarly.

The yeasts for brewery trial one, and for brewery trial two, were collected from previous fermentations stored as a cream, under conditions of refrigeration, and pitched as per the normal procedure for the brewery. The yeast used for fermentation was pitched at a level of 1.6 litres of yeast cream per hectolitre of wort. This is normal yeast handling and pitching procedures for this brewery. For brewery trial one, and of 1.8 litres for brewery trial two.

The zinc content of the yeast was measured before pitching.

Experimental Methods

Laboratory Tests

The sample of the brewery wort obtained was divided up into 2.0 litre aliquots.

For laboratory test 1, seven different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 0.6mg. of zinc chloride, which produced a measured increase of 0.28 mg mineral zinc per litre present in the wort.

Type 2. 40 mg of sacrificial zinc yeast preparation (at 10,500 ppm zinc) which produced a measured increase of 0.24 ppm zinc present in the wort.

Type 3. 160 mg of sacrificial zinc yeast preparation (at 10,500 ppm zinc) which produced a measured increase of 0.805 ppm zinc present in the wort.

Type 4. 8 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which produced a measured increase of 0.26 ppm zinc present in the wort.

Type 5. 16 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.88 ppm measured additional zinc present in the wort.

For laboratory test 2, six different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 0.6 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.16 ppm measured additional zinc in the wort.

Type 2. 30 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.8 ppm measured additional zinc in the wort.

Type 3. 160 mg of dry dead brewers yeast, which corresponds to no measured increase in the zinc content of the wort.

Type 4. 0.6 mg of zinc chloride, which corresponds to 0.15 ppm measured additional zinc in the wort.

Type 5. Addition of 80 mg dry dead brewers yeast plus 0.6 mg zinc chloride which corresponds to 0.15 measured additional zinc in the wort.

For laboratory test 3, six different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 4.6 mg of sacrificial zinc yeast preparation (at 70.000 ppm zinc) which corresponds to 0.26ppm measured additional zinc in the wort.

Type 2. 160mg of dry dead brewers yeast, which corresponds to 0.01 ppm measured increase of zinc in the wort.

Type 3. 35 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which actually corresponds to a measured increase 1.12 ppm of zinc in the wort.

Type 4. 2.5 mg. of zinc chloride, which actually corresponds to a measured increase 0.25 ppm measured increase of zinc in the wort.

Type 5. Addition of 160mg dry dead brewers yeast plus 0.6g zinc chloride, which corresponded to a measured increase of 0.29 ppm zinc in the wort.

Each aliquot was boiled for fifteen minutes. The zinc preparations were added at the start of the boil. The boiling vapours were condensed and returned to the respective lot in order to minimise evaporative loss. The wort preparations were sealed, allowed to cool to 8°C then, pitched with the appropriate quantity of yeast.

Brewery Trials

For brewery trial 1, as for brewery trial 2, one fermenter received the equivalent of 0.30 ppm of additional zinc whilst the other received nothing.

Fermentation

Laboratory Tests one, two and three

Fermentation was carried out in a constant temperature room at approximately 10°C until a density of 3.6°C had been achieved. This is normal for beers that are transferred to lager under conditions of refrigeration so that secondary fermentation and maturation can take place.

Brewery Trials

Fermentation was carried under the standard temperature programme for that particular wort type. The standard and test worts were subject to the same profile.

Measurements

Laboratory tests one, two and three.

Samples were drawn from the wort prior to pitching with yeast and the zinc content was measured.

Fermentation progress was measured by a standard brewing densitometer and recorded in degrees Plato after compensation for temperature effects.

At the beginning of fermentation zinc determinations were carried out on the untreated and treated worts and the pitching yeast. Determinations had previously been carried out on the zinc yeast preparations.

The finished beer in all three trials was analysed using a SCABA "automatic beeranalyser" from PERSTOP ANALYTICAL, SWEDEN, for alcohol concentration as expresses in volume per volume.

Brewery trials

During fermentation samples were drawn at regular intervals, and progress of the fermentation, was measured by the drop in density of the wort, as expressed in degree Balling.

Results and Conclusions

Laboratory Tests 1, 2, and 3

The time taken, for each fermentation to achieve 3.6 degree Plato, which is judged to be when primary fermentation is complete and the alcohol concentrations after two hundred and forty hours of fermentation, are detailed below in table 2 for laboratory test 1, in table 3 for laboratory test 2, and in table 4 for laboratory test 3.

Table 2

In Addition		Zinc Chloride	Zinc Yeast	Zinc Yeast	Zinc Yeast	Zinc Yeast
Zinc Conc. ppm	0.2	0.48	0.44	1.05	0.46	1.08
Hours to achieve 3.6°P	186	168	150	127	110	110
Hours difference from standard	0	-14	-36	-59	-76	-76
% of standard fermentation time	100	90	81	68	59	59
Alcohol by Volume	4.74	4.92	5.05	5.3	5.26	5.3

Table 3

	0	1	2	3	4	5
Addition		Zinc Yeast	Zinc Yeast	Dead yeast	Zinc Chloride	Dead Yeast + zinc chloride
Zinc Conc. ppm	0.06	0.22	0.86	0.06	0.21	0.21
Hours to achieve 3.6°P	175	153	148	186	156	163
Hours difference from standard	0	-20	-24	+10	-14	-2
% of standard fermentation time	100	87	84	106	89	93
Alcohol by Volume	4.86	5.02	5.05	4.93	4.91	4.96

Table 4

	0	1	2	3	4	5
Addition		Zinc Yeast	Dead Yeast	Zinc Yeast	Zinc Chloride	Dead Yeast + Zinc chloride
Zinc Conc. ppm	0.1	0.36	0.11	1.22	0.35	0.39
Hours to achieve 3.6°P	182	162	180	158	177	168
Hours difference from standard	0	-20	-2	-24	-5	-14
% of standard fermentation time	100	89	99	87	97	92
Alcohol by Volume	5.78	5.86	5.81	5.86	5.6	5.74

Conclusions

1. In all fermentation tests where sacrificial zinc yeast was added, fermentation speed was improved in comparison to the standard wort, wort with added zinc chloride, and, when tested, wort with added dead yeast, and wort with added zinc chloride plus dead yeast.

2. In all fermentation tests where sacrificial zinc yeast was added, the standard specific gravity, designated for onward processing of the beer for lagering, was achieved faster than the standard wort, wort with added zinc chloride, and, when tested, wort with added dead yeast, and wort with added zinc chloride plus dead yeast. The time to achieve this degree of fermentation attenuation was at least twenty hours and as great as seventy-six hours less than the standard.

3. Against the test where zinc chloride was added, the sacrificial zinc yeast trials achieved the standard fermentation attenuation at least six and as great as sixty-two hours sooner.

4. Where the zinc addition was at a similar level from mineral zinc (zinc chloride), and biological zinc (sacrificial zinc yeast), the sacrificial zinc yeast experiments were measurably and significantly faster.

5. In all fermentation tests where sacrificial zinc was added, the final concentration of alcohol produced aften ten days, was greater than the standard wort, the wort containing zinc chloride, and when tested, the wort containing inactivated yeast and the wort containing zinc chloride plus inactivated yeast.

Brewery trial 1 and trial 2

Data collected from the brewery fermentation trial number 1 and 2 are displayed in the below table 5. Graphical representation of these data are displayed in figure 1 for brewery trial 1, and in figure 2 for brewery trial .

Table 5

	Trial 1 (primary attenuation limit 3.8)		Trial 2 (primary attenuation limit 3.6)	
Fermentation days	0-Wort	Zinc Yeast	0-Wort	Zinc Yeast
0	11.6	11.6	11.5	11.5
1	10.8	9.6	10.5	10.5
2	9.5	7.8	9	7.5
3	8.8	6	7	6
4	7.6	4.3	5.2	3.5
5	6.7	3.9	4.2	2.1
6	5.7	3.4	3.5	2
7	4.8	2.8	2.8	1.9
8	4.4	2.7	2.6	1.8
9	4	2.7	2.2	1.8

Conclusions

1. The fermentation containing sacrificial zinc yeast was faster than the standard untreated wort.
2. In the fermentation trials where sacrificial zinc yeast was added, the standard specific gravity, 3.8 and 3.6 degrees Balling respectively, designated for onward processing of the beer for lagering was achieved one hundred and forty hours sooner (trial 1), and forty eight hours sooner (trial 2), than the standard untreated wort.

The two beers produced were tasted, by a party of professional brewers and others with professional expertise in beer tasting. The trial beer was judged to be at least as good as the standard and was preferred by many.

The tasters noted that both test beers were particularly lower in "sulphitic" character. This is of particular importance and significance for not only does it indicate a "cleaner" beer it indicates a possibility of advancing the maturation process and thus reducing lager times and costs. At the cellular level, zinc addition according to the invention can show the following stimulatory effects: stabilising proteins and membrane systems, acting as a catalytic centre of essential enzymes (*e.g.* alcohol dehydrogenase, aldolase and acetaldehyde dehydrogenase), enhancing riboflavin synthesis, activating acid and alkaline synthesis, stimulating the uptake of maltose and maltotriose.

EXAMPLE 2 : Residual sugar analysis

After two hundred and forty hours of fermentation of laboratory test the residual sugars in all the beers of test 1 were analysed by gas liquid chromatography. The results are detailed in the below table 6.

Table 6

Experiment	**0**	**1**	**2**	**3**	**4**	**5**
Sugars as mg/100ml		Zinc Chloride	Zinc Yeast	Zinc Yeast	Zinc Yeast	Zinc Yeast
Glucose	0	0	0	0	0	0
Fructose	0	0	0	0	0	0
Sucrose	0	0	0	0	0	0
Maltose	0.69	0.47	0	0	0	0
Maltotriose	0.15	0.09	0	0	0	0
Total	0.84	0.56	0	0	0	0

Conclusions

It is clear from the data presented above that the inclusion of sacrificial zinc yeast permits the fermentation to progress to absolute dryness. That is to say there are no fermentable sugars left in the beer. This is highly significant as it will permit brewers to produce beers free of residual fermentable sugars much quicker that at present. The technical features of the process according to the invention thus lead to more alcohol produced, thereby giving an economic advantage.

EXAMPLE 3 : Quality and quantity of secondary flavour compounds.

After the completion of the fermentations in laboratory test 2 (see the above example 1) the beer was subject G.L.C. analysis to see if the accelerated fermentations affected the quality and quantity of secondary flavour compounds. The results are detailed in the below table 7.

Table 7

Mg/L	**0**	**1**	**2**	**3**	**4**	**5**
	0	Zinc Yeast	Zinc Yeast	Dead Yeast	Zinc chloride	Dead yeast + Zinc chloride
Diacetyl	0.22	0.27	0.29	0.22	0.24	0.24
Pantadione 2.3	0.21	0.25	0.26	0.22	0.22	0.23
Acetadehyde	24.2	25.6	27.9	25.9	24.2	25.5
Ethyl acetate	28.7	25.2	30.9	27.3	28.7	27.8
i-Butanol	7.6	7.5	8.1	7.4	7.6	8
n-Propanol	10.2	10.4	11.4	10.7	10.4	11.2
Amyl acetate	2.8	2.5	3.2	2.7	2.9	2.8
Amyl alcohol	52.1	49.3	52.7	50.9	51.4	52

Conclusions

It is evident from the results above that accelerating the fermentation, by way of sacrificial zinc yeast, has no significant negative effect on the major, secondary, organoleptically active metabolites. Such a negative effect has indeed not been observed in any of the tests and trials performed (see the above example 1). This is particulary striking effect of the process of the invention which allows an accelerated fermentation without negatively affecting the alcohol profile of the product. These conclusions on the quality of the beer produced according to the invention were further confirmed by blind taste panels.
This is a significant finding as it allows a standard beer to be produced at a faster, and therefore cheaper rate.

It will be apparent to those skilled in the art that the process of the present invention which comprises the use of a mineral-rich yeast, and in particular of a zinc-rich yeast, as a fermentation nutrient is a very valuable technical contribution to additive-free brewing. It will also be apparent that the foregoing examples have been for purposes of illustration, and that a number of changes and modifications can be made without departing from the spirit and scope of the invention. The present invention illustrated with a zinc-rich yeast can thus be implemented without undue burden with a yeast rich in any mineral or combination of minerals appropriate to a yeast growth enhancement process, *e.g.* magnesium, manganese.

CLAIMS

1.- Process for alcoholic fermentation, characterized in that it comprises the use of at least one mineral-rich or mineral-enriched yeast as a nutrient source for said fermentation.

2.- Process according to claim 1, characterized in that said at least one mineral-rich or enriched yeast belongs to the *Saccharomyces* genera or to the *Kluyveromyces* genera.

3.- Process according to anyone of claim 1 or 2, characterized in that said at least one mineral-rich or mineral-enriched yeast is prior to use such as obtained by adding about 1,000 to about 200,000 ppm (relative to the weight of the yeast, as measured on a dry weight basis) of a salt of said mineral to a live culture of said micro-organism at a temperature of about 4-40°C, preferably about 25-32°C, at a pH of between about 3.5 to 7.0, preferably about 4.6-6.6, for a period of about 1-24 hours, preferably 2-16 hours so as to allow said micro-organism to incorporate said mineral.

4.- Process according to claim 3, characterized in that said salt is chosen among the group consisting of acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide, perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate or valerate.

5.- Process according to claim 3 or 4, characterized in that said mineral incorporation corresponds to an absorption and/or an adsorption.

6.- Process according to anyone of claims 1 to 5, characterized in that said mineral is a metal capable of altering the metabolism of said fermentation.

7.- Process according to anyone of claims 1 to 6, characterized in that said mineral is chosen among the group consisting of zinc, magnesium and manganese.

8.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast contains before being used a concentration in said mineral ranging from about 1,000 to about 200,000 ppm .

9.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used under a form chosen among the group consisting of a living form and a dead form.

10.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used under a form chosen among the group consisting of a dry form, a liquid form, a frozen form, a freeze-dried form, a paste, a powder.

11.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used by directly adding it at at least one step of said fermentation process.

12.- Process according to claim 11, characterized in that said addition is performed directly into the fermenter, and/or into the boiling vessel, and/or any vessel between the two, and/or into the fermentation micro-organism holding or propagating vessels.

13.- Process according to anyone of the preceding claims, characterized in that said use leads to an increase of at least about 0.05 ppm of the mineral content of the substrate of said fermentation.

14. - Process according to anyone of the preceding claims, characterized in that said alcoholic fermentation can lead to the production of beer.

15.- Process according to anyone of the preceding claims, characterized in that said alcoholic fermentation can lead to the production of an alcohol chosen among the group consisting of whisky or sake as well as fruit, sugar or honey based fermentations, such as wine, brandy, cider, fruit wines, mead, rhum, tequila, industrial alcohols, potable alcohols.

16.- Use of at least one mineral-rich or mineral-enriched yeast as a nutrient source in the production of an alcohol by fermentation.

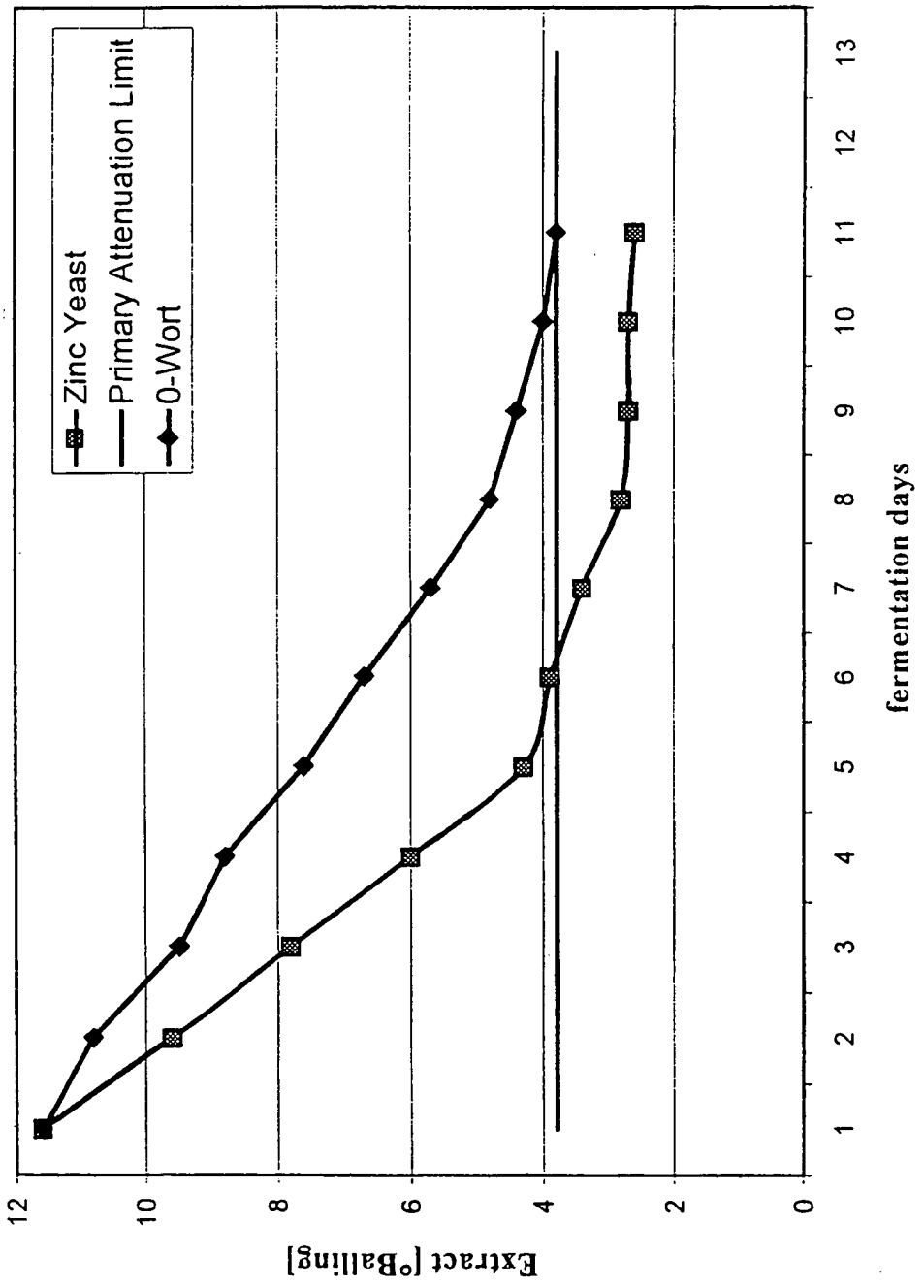
Zinc Yeast
Primary Attenuation Limit
0-Wort
Extract [°Balling]
0
2
4
6
8
10
12
fermentation days
1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 1

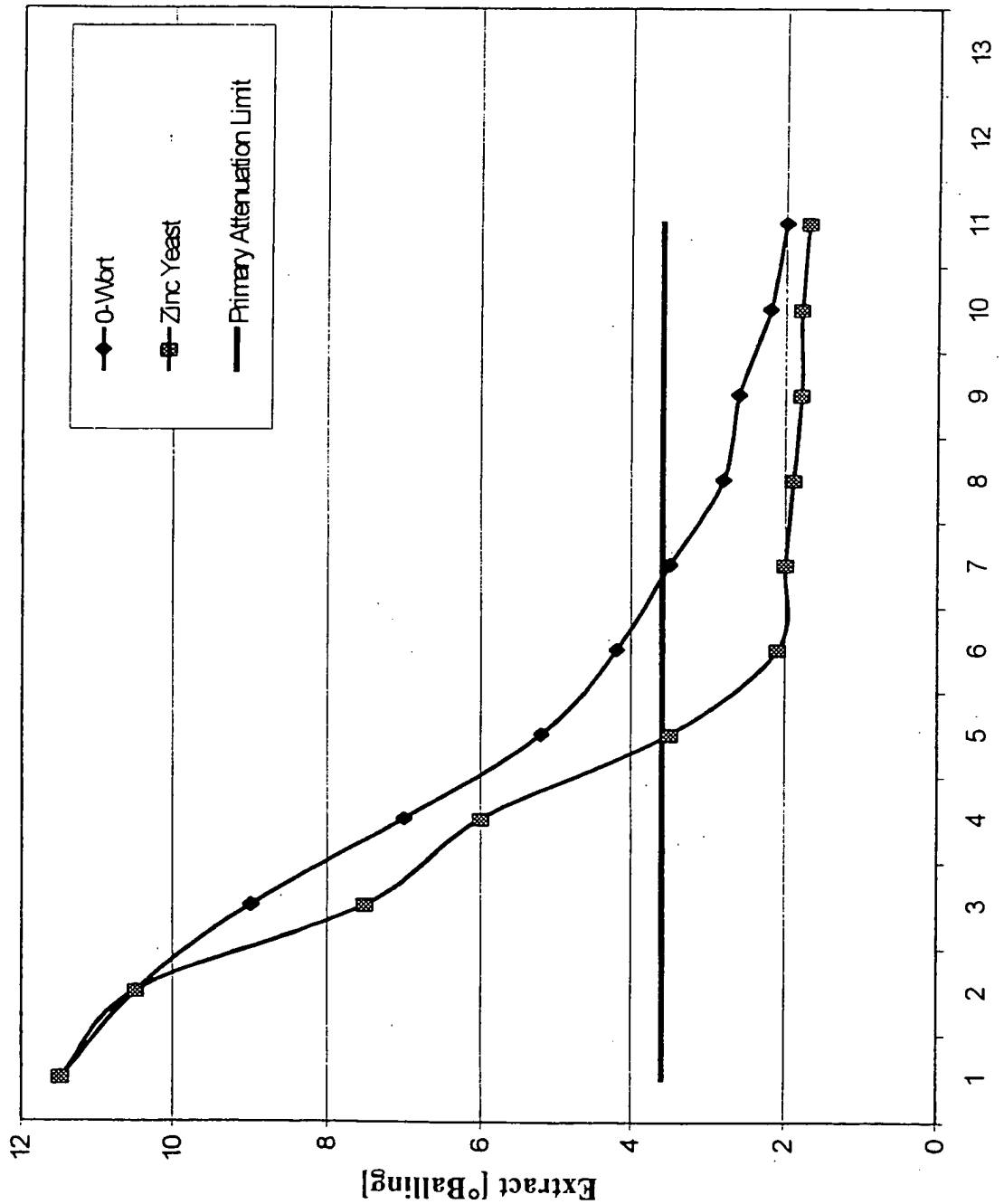
0-Wort
Zinc Yeast
Primary Attenuation Limit
Extract [°Balling]
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
11
12
13
fermentation days

Figure 2

into another beer wort, which is also deficient, would exacerbate fermentation problems.

Other minerals have been shown to be of importance in the course of an alcoholic fermentation. Manganese is thus known to be implicated as a substitute zinc metabolism, and could possibly mitigate some toxic effects associated with high concentrations of zinc. Another example is magnesium which is reported to be important for alcohol efficiency in fermentations. This is particularly a problem for the fermentation of certain substrates where there is an excess of calcium ions present. Calcium is indeed known to be antagonistic to magnesium metabolism and, for example, in beer, calcium is deliberately added in order to control the pH (acidity) and activate some of the enzymes of the malted barley.

For most alcoholic fermentations, there is thus a perceived natural mineral deficit in the substrate, and minerals, in the form of mineral salts such as zinc/manganese/magnesium chloride or sulphate, are generally added directly into the substrate, *e.g.* into the wort at the boiling stage for beer production. The use of such mineral salts, whilst relatively effective, conflicts with the desire by some industrialists to produce additive free alcohols.

Alternatives to the addition of mineral salts have thus been proposed in the past decades. These include pre-loading the fermentation yeast with a metal in such a way that the metal is hardly released from the cell body of the fermentation yeast during the fermentation process, or using ash trub or acid extracts of spent grains or hop trub so as to make use of the trace elements they contain. But all these alternatives are, in terms of quantity and quality of alcoholic fermentation production, at the best only substantially equivalent to the initial solution of directly adding mineral

period of about 1-20 or 24 hours, preferably 2-16 hours so as to allow said yeast to incorporate, absorb and/or adsorb the mineral(s). Any salt, *e.g.* acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide, perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate or valerate is appropriate. Comparative assays can be performed by the person skilled in the art to determine the most efficient mineral source. Said mineral incorporation can correspond to an absorption and/or an adsorption. When incorporated, said mineral may remain as a mineral and/or be transformed into a salt and/or an organic form. It has to pointed out that the efficacy of the use according to the invention is not necessarily directly and solely dependent on the resultant mineral concentration of the substrate : bioavailability has also to be taken into account. In another embodiment, said at least one mineral-rich or enriched yeast is a commercially available product, *e.g.* a product from the Danstar Ferment A.G. Mineral Enriched Yeast range.

Said yeast is advantageously rich in, or enriched in at least one mineral which is capable of altering the metabolism of an alcoholic fermentation. A capacity of altering the metabolism of an alcoholic fermentation can be easily assessed by the person skilled in the art, *e.g.* by comparing the growth level of the fermentation micro-organism, and/or the rate of fermentation, and/or the secondary metabolites concentrations and/or the flavour profile, in the presence and in the absence of the mineral candidate under standard appropriate laboratory conditions. The word "mineral" herein also comprises oligoelements. Such a mineral is preferably a metal, and most preferably a divalent metal. It is advantageously chosen among the group consisting of zinc, magnesium,

Another advantage lies in the fact that said use can be performed, as desired, at any step of the fermentation process. A simple direct addition of said at least one mineral-rich or enriched yeast at at least one step of the fermentation process is efficient. It may thus be added directly into the boiling vessel, and/or the fermenter, and/or any vessel between the two, and/or into the vector micro-organism holding or propagating vessels. For example, in beer production, the addition to the wort can be performed during alcohol production process or fermentation micro-organism propagation process, before or after boiling.

Said at least one mineral-rich or enriched yeast can thus be added directly to the wort so that it is killed during the wort boiling stage. It may be also added to the cooled wort prior to, during or after yeast pitching. Preferably said at least one mineral-rich or enriched yeast is added to the boiling wort. Advantageously, said use according to the invention is performed so that it leads to an increase of at least about 0.05 ppm of the mineral content of the substrate of said fermentation . The fermented substrate itself may be distilled or not.

The use according to the invention is particularly efficient in that it accelerates alcoholic fermentation velocity greater than when the mineral concentration is raised by the addition of the equivalent concentration of mineral when derived from a mineral salt. A synergetic effect can moreover be outlined when comparing to the addition of mineral salt on one hand and dead yeast on the other hand (see *e.g.* laboratory tests 2 and 3 of example 1 for zinc). The fermentation duration needed therefore decreases (see examples below). The limit to primary fermentation is achieved faster, significantly in comparison to when the equivalent

in brewery trial 2 is deposited with the Technical University of Munich-Weihenstephan Hefebank, and designated as strain number W 34/70. The strain used in brewery trial 1 is deposited with the Technical University of Munich-Weihenstephan Hefebank, and designated as strain number W 120.

The yeast used for all laboratory trials was obtained, when needed, in the form of a cream from the appropriate brewery. The cream was centrifuged in a SORVAL RC5B centrifuge at 2700g for 10 minutes and the supernatant was discarded. The yeast paste was weighed and resuspended in cooled wort, aerated and then added directly to the fresh worts. The zinc content of the yeast was measured before pitching.

Media

Laboratory Test Fermentations

The wort used was obtained from three commercial breweries in Germany. In test number one the wort is used to make their "helle" type beer. In test number two the wort is used to make their "festbier". In test number three the wort is used to produce "pilsner" type beer. In all cases the wort was collected at the end of the boil and was therefore hopped to the normal level of that product for that particular beer. The worts had not been treated in the respective brewery in any way to alter the natural level of zinc. The worts were boiled for fifteen minutes before being cooled to the fermentation temperature, 10°C, and pitched with fermentation yeast.

Brewery Trials

Two brewery trials were carried out in two commercial breweries in Germany. The brewery in trial one was the Brauerei Kreiger, 944505 Landau, D. Isar, Bavaria, Germany. During a normal commercial production phase, two consecutive "helle" type worts, number 22 and 23, produced on 20th and 21st of April 1999 respectively, were designated for experimental observation. They were produced from the same recipe, one immediately after the other, from the same malt and hop stocks, and brewing water. 110 hectolitres of wort was collected from each brew.

The brewery in trial two was, the Privatbrauerei Kitzmann, Kitzmann Bräu KG, Südliche Stadmauerstrasse 25, 91954 Erlangen Bavaria, Germany. During a normal commercial production phase, one wort, designated brew number 120, produced on the 26th of April 1999, containing 286 litres of wort at 11.6° Balling, was separated into two fermenters each containing one hundred and forty-three hectolitres of "pilsner" wort. Both temperature profiles of the fermentations were as per normal for that brewery for that beer type.

Zinc Preparation

Laboratory tests

Mineral zinc, when used, was added in the form of the salt zinc chloride. This salt is used extensively by breweries throughout the World.

Zinc measurements on the wort and yeast samples were carried out by atomic absorption spectrometry as per the MEBAK standard brewery analytical procedures, see *e.g* Lutz,A.: Bestrmmung, Vorkommen und

Verhalten von Kontaminationen durch verschiede umweltrelevante Spurenelemente in Bereich der Brauerei, Dissertation TU München, (1996), S21 ff.

Preparation of Sacrificial zinc Yeast.

A sacrificial zinc yeast can be prepared by the person skilled in the art by any method convenient for producing a zinc-rich yeast. Standard methods use the incorporation, absorption and/or adsorption of zinc by the yeast. It should be noted that to implement the present invention, the yeast could be living or dead.

The preparations used in the trials herein reported were produced in some manner as per the following method.

Zinc, at a concentration of between 1,000 and 200,000 ppm (relative to the weight of the yeast or yeast fraction, as measured on a dry weight basis), in the form of zinc sulphate, chloride, acetate, phosphate, or some other appropriate zinc form is added to a live or dead culture of *S. cerevisae* at a temperature of about 4 to about 40° Celsius (preferably of about 25-32°C) at pH of between about 3.5 to about 7 (preferably about 4.6 to about 6.6), for a period of 1 to 20 or 24 hours so as to allow the culture to incorporate, absorb and/or adsorb the zinc.

Two base dry zinc yeast preparations were used in the trials;

Preparation one contained 10,500 ppm mineral zinc

Preparation two contained 70,000 ppm mineral zinc

Other zinc yeast preparations are also commercially available from Danstar Ferment AG, 20 Alpenstrasse, 6301 ZUG, Switzerland (MEY Zn 50).

All the zinc preparations were added to the boiling worts, at the start of boiling.

In summary, the state of zinc in the yeasts and worts in the three laboratory tests and two brewery trials is as follows in :

Table 1

	Test 1	Test 2	Test 3	Trial 1	Trial 2
Wort ppm	0.2	0.06	0.1	0.06	0.1
Fermentation Yeast mg/100g dry	5.9	1.3	4.1	1.3	5

Fermentation Yeast Preparation

A portion of the test yeast, 30 g of the paste, was re-suspended in 250 millilitre of boiled wort and aerated for approximately five minutes by way of a magnetic stirrer. The yeast preparation was then divided into seven equal aliquots and pitched into the appropriate test wort.

Brewery trial one and brewery trial two were conducted similarly.

The yeasts for brewery trial one, and for brewery trial two, were collected from previous fermentations stored as a cream, under conditions of refrigeration, and pitched as per the normal procedure for the brewery. The yeast used for fermentation was pitched at a level of 1.6 litres of yeast cream per hectolitre of wort. This is normal yeast handling and pitching procedures for this brewery. For brewery trial one, and of 1.8 litres for brewery trial two.

The zinc content of the yeast was measured before pitching.

Experimental Methods

Laboratory Tests

The sample of the brewery wort obtained was divided up into 2.0 litre aliquots.

For laboratory test 1, seven different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 0.6mg. of zinc chloride, which produced a measured increase of 0.28 mg mineral zinc per litre present in the wort.

Type 2. 40 mg of sacrificial zinc yeast preparation (at 10,500 ppm zinc) which produced a measured increase of 0.24 ppm zinc present in the wort.

Type 3. 160 mg of sacrificial zinc yeast preparation (at 10,500 ppm zinc) which produced a measured increase of 0.805 ppm zinc present in the wort.

Type 4. 8 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which produced a measured increase of 0.26 ppm zinc present in the wort.

Type 5. 16 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.88 ppm measured additional zinc present in the wort.

For laboratory test 2, six different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 0.6 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.16 ppm measured additional zinc in the wort.

Type 2. 30 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.8 ppm measured additional zinc in the wort.

Type 3. 160 mg of dry dead brewers yeast, which corresponds to no measured increase in the zinc content of the wort.

Type 4. 0.6 mg of zinc chloride, which corresponds to 0.15 ppm measured additional zinc in the wort.

Type 5. Addition of 80 mg dry dead brewers yeast plus 0.6 mg zinc chloride which corresponds to 0.15 measured additional zinc in the wort.

For laboratory test 3, six different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 4.6 mg of sacrificial zinc yeast preparation (at 70.000 ppm zinc) which corresponds to 0.26ppm measured additional zinc in the wort.

Type 2. 160mg of dry dead brewers yeast, which corresponds to 0.01 ppm measured increase of zinc in the wort.

Type 3. 35 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which actually corresponds to a measured increase 1.12 ppm of zinc in the wort.

Type 4. 2.5 mg. of zinc chloride, which actually corresponds to a measured increase 0.25 ppm measured increase of zinc in the wort.

Type 5. Addition of 160mg dry dead brewers yeast plus 0.6g zinc chloride, which corresponded to a measured increase of 0.29 ppm zinc in the wort.

Each aliquot was boiled for fifteen minutes. The zinc preparations were added at the start of the boil. The boiling vapours were condensed and returned to the respective lot in order to minimise evaporative loss. The wort preparations were sealed, allowed to cool to 8°C then, pitched with the appropriate quantity of yeast.

Brewery Trials

For brewery trial 1, as for brewery trial 2, one fermenter received the equivalent of 0.30 ppm of additional zinc whilst the other received nothing.

Fermentation

Laboratory Tests one, two and three

Fermentation was carried out in a constant temperature room at approximately 10°C until a density of 3.6°C had been achieved. This is normal for beers that are transferred to lager under conditions of refrigeration so that secondary fermentation and maturation can take place.

Brewery Trials

Fermentation was carried under the standard temperature programme for that particular wort type. The standard and test worts were subject to the same profile.

Measurements

Laboratory tests one, two and three.

Samples were drawn from the wort prior to pitching with yeast and the zinc content was measured.

Fermentation progress was measured by a standard brewing densitometer and recorded in degrees Plato after compensation for temperature effects.

At the beginning of fermentation zinc determinations were carried out on the untreated and treated worts and the pitching yeast. Determinations had previously been carried out on the zinc yeast preparations.

The finished beer in all three trials was analysed using a SCABA "automatic beeranalyser" from PERSTOP ANALYTICAL, SWEDEN, for alcohol concentration as expresses in volume per volume.

Brewery trials

During fermentation samples were drawn at regular intervals, and progress of the fermentation, was measured by the drop in density of the wort, as expressed in degree Balling.

Conclusions

It is evident from the results above that accelerating the fermentation, by way of sacrificial zinc yeast, has no significant negative effect on the major, secondary, organoleptically active metabolites. Such a negative effect has indeed not been observed in any of the tests and trials performed (see the above example 1). This is particulary striking effect of the process of the invention which allows an accelerated fermentation without negatively affecting the alcohol profile of the product. These conclusions on the quality of the beer produced according to the invention were further confirmed by blind taste panels.

This is a significant finding as it allows a standard beer to be produced at a faster, and therefore cheaper rate.

It will be apparent to those skilled in the art that the process of the present invention which comprises the use of a mineral-rich yeast, and in particular of a zinc-rich yeast, as a fermentation nutrient is a very valuable technical contribution to additive-free brewing. It will also be apparent that the foregoing examples have been for purposes of illustration, and that a number of changes and modifications can be made without departing from the spirit and scope of the invention. The present invention illustrated with a zinc-rich yeast can thus be implemented without undue burden with a yeast rich in any mineral or combination of minerals appropriate to a yeast growth enhancement process, *e.g.* magnesium, manganese.

CLAIMS

1.- Process for alcoholic fermentation, characterized in that it comprises the use of at least one mineral-rich or mineral-enriched yeast as a nutrient source for said fermentation.

2.- Process according to claim 1, characterized in that said at least one mineral-rich or enriched yeast belongs to the *Saccharomyces* genera or to the *Kluyveromyces* genera.

3.- Process according to anyone of claim 1 or 2, characterized in that said at least one mineral-rich or mineral-enriched yeast is prior to use such as obtained by adding about 1,000 to about 200,000 ppm (relative to the weight of the yeast, as measured on a dry weight basis) of a salt of said mineral to a live culture of said micro-organism at a temperature of about 4-40°C, preferably about 25-32°C, at a pH of between about 3.5 to 7.0, preferably about 4.6-6.6, for a period of about 1-24 hours, preferably 2-16 hours so as to allow said micro-organism to incorporate said mineral.

4.- Process according to claim 3, characterized in that said salt is chosen among the group consisting of acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide, perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate or valerate.

5.- Process according to claim 3 or 4, characterized in that said mineral incorporation corresponds to an absorption and/or an adsorption.

6.- Process according to anyone of claims 1 to 5, characterized in that said mineral is a metal capable of altering the metabolism of said fermentation.

7.- Process according to anyone of claims 1 to 6, characterized in that said mineral is chosen among the group consisting of zinc, magnesium and manganese.

8.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast contains before being used a concentration in said mineral ranging from about 1,000 to about 200,000 ppm .

9.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used under a form chosen among the group consisting of a living form and a dead form.

10.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used under a form chosen among the group consisting of a dry form, a liquid form, a frozen form, a freeze-dried form, a paste, a powder.

11.- Process according to anyone of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used by directly adding it at at least one step of said fermentation process.

12.- Process according to claim 11, characterized in that said addition is performed directly into the fermenter, and/or into the boiling vessel, and/or any vessel between the two, and/or into the fermentation micro-organism holding or propagating vessels.

13.- Process according to anyone of the preceding claims, characterized in that said use leads to an increase of at least about 0.05 ppm of the mineral content of the substrate of said fermentation.

14. - Process according to anyone of the preceding claims, characterized in that said alcoholic fermentation can lead to the production of beer.

15.- Process according to anyone of the preceding claims, characterized in that said alcoholic fermentation can lead to the production of an alcohol chosen among the group consisting of whisky or sake as well as fruit, sugar or honey based fermentations, such as wine, brandy, cider, fruit wines, mead, rhum, tequila, industrial alcohols, potable alcohols.

16.- Use of at least one mineral-rich or mineral-enriched yeast as a nutrient source in the production of an alcohol by fermentation.

ATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
CP/FP966		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IB 99/00924	21/05/1999	23/05/1998
Applicant		
DANSTAR FERMENT A.G. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/IB 99/00924

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P7/06 C12C11/00 C12G1/00 C12G3/00 C12G3/02
C12N1/16 C12N1/18 C12C11/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P C12C C12G C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 128, no. 2, 12 January 1998 (1998-01-12) Columbus, Ohio, US; abstract no. 12760, BAKOYIANIS, V. ET AL: "Comparative Study of Kissiris,. gamma.- Alumina, and Calcium Alginate as Supports of Cells for Batch and Continuous Wine-Makin at Low Temperatures" XP002111347 abstract & J. AGRIC. FOOD CHEM. (1997), 45(12), 4884-4888 , --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 August 1999	20/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Douschan, K

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INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/IB 99/00924

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 116, no. 9, 2 March 1992 (1992-03-02) Columbus, Ohio, US; abstract no. 82160, ICONOMOU, L. ET AL: "The promotion of molasses alcoholic fermentation using Saccharomyces cerevisiae in the presence of. gamma.- alumina" XP002111348 abstract & APPL. BIOCHEM. BIOTECHNOL. (1991), 31(1), 83-96 , ---	1-16
Y	CHEMICAL ABSTRACTS, vol. 96, no. 19, 10 May 1982 (1982-05-10) Columbus, Ohio, US; abstract no. 161190, KAKO HONSHA K. K., JAPAN: "Mineral - rich health food materials" XP002111349 abstract & JP 57 018969 A (KAKO HONSHA K. K., JAPAN) 1980 ---	1-16
Y	WO 93 01298 A (KOUTINAS, A. ET AL.) 21 January 1993 (1993-01-21) the whole document -----	1-16

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

In. ational Application No
PCT/IB 99/00924

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 57018969 A	30-01-1982	JP 1136117 C	28-02-1983
		JP 57026742 B	07-06-1982
WO 9301298 A	21-01-1993	DE 69218758 D	07-05-1997
		EP 0565647 A	20-10-1993
		GR 3023972 T	31-10-1997